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The acidity and self-catalyzed lactonization of L-gulonic acid: Thermodynamic, kinetic and computational study

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ABSTRACT

Lactonization and proton dissociation of sugar acids take place simultaneously in acidic aqueous solutions. The protonation-deprotonation processes are always fast, whilst the formation and hydrolysis of γ- and δ-lactones are usually slower. Thus, both thermodynamic and kinetic information are required for the complete understanding of these reactions. The protonation constant (K_p) of L-gulonate (Gul^-) was determined from potentiometric and polarimetric measurements, while the individual lactonization constants ($K_{L,\gamma}$ and $K_{L,\delta}$) for L-gulonic acid (HGul) were obtained *via* ^{13}C NMR experiments. The applicability of this method was proven by measuring these well-known constants for D-gluconic acid (HGluc) and by comparing them to literature data. L-gulonic acid γ-lactone (γ-HGul) has remarkable stability in contrast with δ-HGul as well as γ- and δ-HGluc. The polarimetric measurement implies that the main factor responsible for the enhanced stability of γ-HGul is that its hydrolysis is much slower than that of δ-HGul. This higher stability of the γ-HGul ring over its δ-isomer was also confirmed by quantum chemical calculations. A new confirmed feature of the reaction is that in parallel to H_3O^+, HGul also catalyzes the formation and reverse hydrolytic processes of γ-HGul, similarly to other general acid catalysts.

1. Introduction

Lactones are intramolecular esters of hydroxycarboxylic acids occurring in nature usually as five- (γ) and six-membered (δ) cyclic compounds. They serve as components of various aromas and odors, hence, they are utilized in the cosmetic and food industries. Additionally, important synthetic applications are also known (*e.g.*, ε-caprolactone).

L-gulonic acid and its γ-lactone (HGul and γ-HGul, Scheme 1) are intermediates in the biosynthesis of L-ascorbic acid in mammals [1]. The enzymatic reactions involve the formation of gulonic acid from D-glucuronic acid as discussed in detail in Refs. [2–8]. Nowadays, the industrial production of ascorbic acid is based on the Reichstein process applying the readily available D-glucose instead of D-glucuronic acid [9]. Attempts were also made to obtain ascorbic acid directly from γ-HGul or its 3,5-*O*-benzylidene derivative [10]. Furthermore, the diol and diisocyanate derivatives of γ-HGul were employed to synthetize polyurethanes [11].

Lactones are formed *via* acid catalysis as follows from the mechanism of the Fischer-Speier esterification [12]. A pioneering work related to the lactonization of HGul and other aldonic acids was carried out by Levene and Simms [13,14]. The five- and six-membered lactones were found to be formed for D-gulonic, D-heptagluconic, D-galactonic, D-gluconic and D-mannonic acids. The authors observed that the δ-lactone was more dextrorotatory, while the γ-lactone was more levorotatory for the first three aldonic acids. On the other hand, gluconic and mannonic acids could only be converted into dextrotatory lactones.

These observations are formulated in Hudson's lactone rule [15], which stipulates that when the OH group involved in the ring closure lies on the right side on the Fischer projection of the acid, the lactone will be more dextrorotatory, otherwise it will be more levorotatory.

HGul is expected to be a stronger acid than caproic acid owing to the presence of electron withdrawing OH functional groups along the aliphatic backbone. The protonation constant, K_p, of Gul is defined as:

$$K_p = \frac{1}{K_a} = \frac{[HGul]\cdot c^o}{[H^+][Gul^-]} \quad (1)$$

where K_a is the acid dissociation constant of HGul and c^o means the

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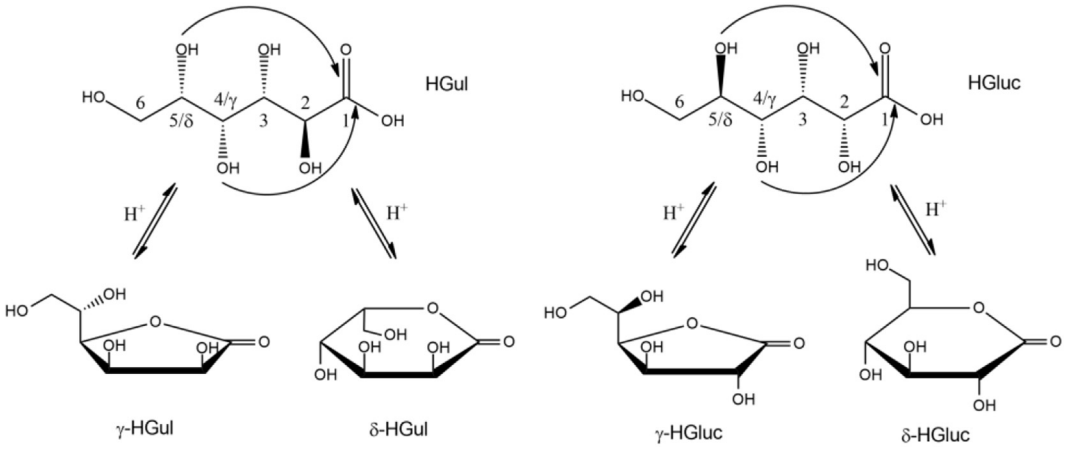

Scheme 1. Left side: structural formulae of L-gulonic acid (HGul) and its γ- and δ-lactones (γ-HGul, δ-HGul). Arrows indicate the OH groups whose attack on the C1 carbon atom yields the five- or six-membered lactone. Right side: the same for D-gluconic acid (HGluc) are shown for comparison.

standard molar concentration, 1 mol dm^{-3}. At infinite dilution, log K_p^0 of HGul at 25 °C was determined to be 3.67 [13]. The value of log K_p was determined to be 3.48 (I = 0.1 M $NaClO_4$, *via* potentiometry) [16] as well as 3.20 and 3.19 (I = 1 M NaCl, *via* ^{1}H and ^{13}C NMR spectroscopies) [17] at 25 °C. For comparison, the log K_p of the caproate ion (same as the pK_a of caproic acid) was reported to be 4.85 [18].

Contrary to gulonic acid, the industrially more important gluconic acid (HGluc, Scheme 1) was studied in great detail previously. The log K_p^0 of HGluc was reported to be 3.77–3.92 [19–21] at 25 °C. At 1 M ionic strength, log K_p was determined to be 3.30–3.63 [21–24] *via* potentiometry and 3.24 [25] *via* ^{13}C NMR spectroscopy, respectively.

The work of Levene and Simms is the only one concerning the lactonization of HGul. The initial rate of δ-HGul formation was eight times higher compared to that of γ-HGul at 25 °C [13].

By Baldwin's rules [26], both the reactions resulting the γ- or the δ-lactone (*i.e.*, the 5-*exo-trig* and the 6-*exo-trig* types of ring closure) are favoured. The larger stability of the γ-lactone corresponds to the qualitative finding that an *exo* double bond stabilizes the five-membered ring [27].

The individual lactonization constants of the two isomers are expressed as:

$$K_{L,\gamma} = \frac{[\gamma\text{–HGul}]}{[\text{HGul}]} \quad (2)$$

$$K_{L,\delta} = \frac{[\delta\text{–HGul}]}{[\text{HGul}]} \quad (3)$$

These equilibrium constants can be estimated from the initial rate coefficients of the lacton formation and reverse hydrolysis reported in Ref. [13]. Accordingly, log $K_{L,\gamma} \approx 0.60$ and log $K_{L,\delta} \approx -0.49$. For HGluc, numerous works give quantitative description of the δ-lactonization reaction. The thermodynamic lactonization constant, log $K_{L,\delta}^0$, was calculated to be −0.95 [20] and −0.81 [21]. Depending on the ionic strength and experimental technique used, log $K_{L,\delta}$ ranges from −1.15 to −0.54 [19,21,28–31]. On the other hand, log $K_{L,\gamma}$ was reported to be −0.59 [19] and −0.62 [29]. Clearly, the difference in the stability of the gluconolactones is much smaller compared to that of the gulonolactones.

Contrary to HGluc, quantitative studies for the lactonization reactions of HGul are sporadic. Thus, the available data need to be extended to provide better understanding of the protonation, lactonization equilibria and the lactonization kinetics of this aldonic acid. In this study, we report on the protonation and individual lactonization constants obtained from potentiometric and ^{13}C NMR measurements. The accuracy of the protonation and lactonization constants of HGul were checked by determining these constants also for HGluc and comparing them with literature data. Additionally, polarimetric experiments and quantum chemical calculations were carried out to characterize the lactonization kinetics and the possible structures of the lactones of HGul present in acidic solutions.

2. Experimental

2.1. Reagents and solutions

All samples were prepared with deionized water (Merck Millipore Milli-Q) and the ionic strength of the solutions was adjusted to 1 M with NaCl (a. r. grade, Molar Chemicals). Stock solutions of HCl were made by volumetric dilution of cc. HCl (a. r. grade, Scharlab), and were standardized against $KHCO_3$. Concentrated (≈50% w/w), carbonate-free NaOH liquor was prepared in-house from pellets (a. r. grade, Analar Normapur) according to the procedure reported previously [32]. The caustic then was brought to volume and the stock solution and it was standardized against HCl solution.

L-gulonic acid γ-lactone (95%, Aldrich), D-gluconic acid δ-lactone (≥99%, Sigma) and sodium D-gluconate (≥99%, Sigma) were used without further purification. The purity of γ-HGul and δ-HGluc was checked by their ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra (0.2 M lactone in 100% V/V D_2O, see Figures S1–S4 in the Electronic Supporting Information). Accordingly, contaminants having peak area larger than 1% were not detected. It has to be mentioned that contrary to γ-HGul, large portion of δ-HGluc underwent hydrolysis even in D_2O (Figures S3 and S4). The solutions of D-gluconate were made directly from the sodium salt, while those of L-gulonate were prepared by neutralizing the lactone with NaOH until pH ≈ 7–8. The completion of ring-opening reaction was confirmed by the ^{1}H and ^{13}C NMR spectra.

2.2. Potentiometric titrations

Potentiometric measurements were carried out with a Metrohm Titrando 888 automatic titration instrument, whilst the cell potentials were recorded with a Jenway 3540 Bench Conductivity/pH Meter using a SenTix 62 combined glass electrode (from WTW). All samples were stirred and thermostated to T = (25.0 ± 0.1) °C during the titrations. To minimize the incidental dissolution of CO_2, N_2 atmosphere was applied.

The calibration of the electrode was performed by titrating 70 cm^3 solution of 0.02 M NaOH with 0.2 M malonic acid (at 1 M ionic strength). The intercept and slope of the electrode were calculated utilizing the pHCali program [33], and the calibration curve was found to be Nernstian.

To avoid problems associated with the lactonization of HGul and

HGluc in acidic medium, measurements for determining K_p were started in the alkaline region. In all cases, 70 cm^3 of solutions containing $[NaGluc]_{T,0}$ = 0.10–0.30 M NaGluc or NaGul were titrated with $[HCl]_{T,0}$ = 0.25–1.0 M, and the data were evaluated with the PSEQUAD program [34]. (For the component X, hereafter the total concentration is denoted as $[X]_{T,0}$ (titrations) or $[X]_T$ (polarimetric and NMR measurements), while [X] represents the equilibrium concentration.)

2.3. NMR measurements

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 MHz NMR spectrometer equipped with a 5 mm inverse broadband probe head furnished with *z* oriented magnetic field gradient capability. The magnetic field was stabilized by locking it to D_2O prior to measurements. The temperature was kept at T = (25 ± 1) °C during all spectra acquisitions. 64–128 as well as 256–8192 interferograms were collected to obtain ^1H and ^{13}C spectra, respectively.

The geminal ($^2J_{H,H}$) or vicinal ($^3J_{H,H}$) coupling constants for γ-HGul obtained in D_2O are presented in Table S1. These parameters were previously reported for the spectroscopically equivalent D-gulonic acid γ-lactone [35]. The agreement is within 0.5 Hz except for $^3J_{4,5}$, being the deviation as much as 3.1 Hz.

The lactonization reactions were performed in excess HCl. Time dependence of the ^{13}C NMR spectra were followed in a solution containing $[NaGul]_T$ = 0.34 M, $[HCl]_T$ = 0.37 M and 20% V/V D_2O. The equilibrium measurements were undertaken for solutions with $[Gul^-]_T$ = 0.40–0.42 M and $[HCl]_T$ = 0.44–0.50 M as well as $[Gluc^-]_T$ = 0.40 M and $[HCl]_T$ = 0.46–0.59 M. The completion of the reaction was checked periodically by polarimetry.

For HGluc, the lactone formation was found to be reversible [19–21,28–31]. In order to check the reversibility of lactonization of HGul, a solution containing 0.42 M NaGul was acidified with 0.63 M HCl, and the equilibrium solution was re-neutralized with 0.63 M NaOH. By the ^1H and ^{13}C NMR spectra (Figures S5 and S6), no irreversible side reactions were observed.

Having reached the equilibrium, 20% V/V D_2O was added to all solutions prior to spectra acquisition resulting dilution ratio of 0.8 and I = 0.8 M.

2.4. Polarimetric measurements

The optical rotation of the samples was recorded with an Optech PL1 polarimeter equipped with a sodium lamp and having an accuracy of ± 0.05°. The length of the light path was 200 mm. All experiments were performed at T = (25 ± 2) °C.

The specific rotations of L-Gul^-, γ-L-HGul and D-$Gluc^-$ were determined by calibration at 1 M ionic strength. These values with their triple standard errors (*SE*, given in parentheses) are as follows: −13.5(3)°, 57.8(3)° and 13.0(3)°. Previously, specific rotation of −12.8° was reported for L-Gul^- [17], while −55.0° was obtained for γ-D-HGul [13]. For D-$Gluc^-$, values ranging between 12.0° and 15.6° [19,20,28,36] were reported.

To determine the specific rotations of L-HGul and D-HGluc, solution series with $[Gul^-]_T$ = 0.31 M and $[HCl]_T$ = 0.03–0.35 M as well as with $[Gluc^-]_T$ = 0.40 M and $[HCl]_T$ = 0.03–0.49 M were prepared. Batch pH measurements were also carried out with a glass electrode (SenTix, calibrated against commercial buffers in the pH range of 1–10). Both optical rotation and pH readings were undertaken simultaneously and right after the sample preparation. The K_p was calculated with the aid of the OriginLab software [37].

Of the four samples used for the equilibrium studies (see the previous section), the one containing $[Gul^-]_T$ = 0.42 M and $[HCl]_T$ = 0.46 M was used to follow the lactonization kinetics of HGul. The optical rotation of this solution was determined in regular time intervals, and the experimental data were evaluated by means of the ChemMech program [38].

It should be emphasized again that the aim of all experiments concerning $Gluc^-$ was to validate the methods used to determine the equilibrium constants for Gul^-.

2.5. Quantum chemical calculations

Geometry optimizations for γ-HGul and δ-HGul were carried out using the M11 range-separated hybrid meta-GGA DFT functional [39] coupled with the def2-TZVP [40] basis set. The recently developed M11 DFT functional was shown to provide excellent performance for main-group energies, proton and electron affinities, barrier heights, bond dissociation and noncovalent interaction energies.

Calculations were performed first in vacuum, then in the framework of implicit water molecules. Solvent effects were taken into account by applying the Conductor-like Polarizable Continuum Model (CPCM) [41] using the default set of radii from the Universal Force Field (UFF) [42]. To find the respective conformers, optimizations were augmented with conformational analysis, that is, the dihedral angle was systematically changed by 60° along the freely-rotating C–C bonds. All calculations were carried out using the Gaussian 09 software package [43].

3. Results and discussion

3.1. Protonation of L-gulonate

The potentiometric measurements for the gulonate-containing solutions are shown in Fig. 1. The four independent titrations were fitted simultaneously, and the ionic product of water, pK_w, was taken as 13.76 [44]. To characterize the goodness of the fit, the so-called fitting parameter (*FP*) was used, which can be defined as:

$$FP = \sqrt{\frac{\sum_{i=1}^{n} (Y_{i,calc} - Y_{i,meas})^2}{n - k}} \quad (4)$$

where $Y_{i,calc}$ and $Y_{i,meas}$ are the i^{th} calculated and measured data (cell potential or optical rotation), n means the number of measured points, and k denotes the number of the fitted parameters. For the titrations, the *FP* was 0.7 mV. The determined stability constants with their triple

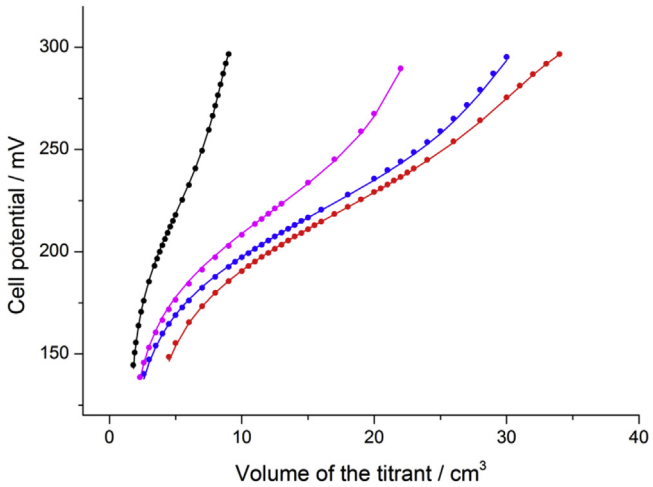

Fig. 1. Potentiometric titration curves for Na-L-gulonate (Gul^-). Experimental conditions: T = (25.0 ± 0.1) °C, I = 1 M (NaCl), V_0 = 70 cm^3. Analytical concentrations of Gul^- and of the titrant: $[Gul^-]_{T,0}$ = 0.097 M and $[HCl]_{T,0}$ = 0.972 M (black); 0.294 M and 0.972 M (magenta); 0.196 M and 0.496 M (blue); 0.097 M and 0.243 M (red). The titrations started in the alkaline region with $[OH^-]_{T,0}$ = 0.01–0.02 M. Symbols and solid lines represent the measured and calculated data, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Protonation (log K_p), γ- and δ-lactonization constants ((log $K_{L,\gamma}$, log $K_{L,\delta}$) for L-gulonate (Gul^-) and D-gluconate ($Gluc^-$). Data correspond to T = 25 °C unless indicated differently. Additionally, the ± 3 *SE* values are given in parentheses.

Reaction	Background el.	Stability constant	Method[a]	Ref.
$H_3O^+ + Gul^- = HGul + H_2O$	$I \rightarrow 0$	$\log K_p = 3.67$	POT	13
	0.1 M $NaClO_4$	3.48(6)	POT	16
	1 M NaCl	3.325(4)[b]	POT	p. w.[c]
	1 M NaCl	3.33(3)[d]	POT	p. w.
	1 M NaCl	3.14(9)	POL	17
	1 M NaCl	3.19(9)	^{13}C NMR	
HGul = γ-HGul + H_2O	0.8 M NaCl	$\log K_{L,\gamma} = 0.79(3)$	^{13}C NMR	p. w.
	1 M NaCl	0.73(3)	POL	p. w.
	not indicated	0.60[e]	POL	13
HGul = δ-HGul + H_2O	0.8 M NaCl	$\log K_{L,\delta} = -0.91(6)$	^{13}C NMR	p. w.
	not indicated	−0.49[e]	POL	13
$H_3O^+ + Gluc^- = HGluc + H_2O$	$I \rightarrow 0$	$\log K_p = 3.85$	POL	19
	$I \rightarrow 0$	3.77(6)	POL	20
	$I \rightarrow 0$	3.92(10)	POT	21
	1 M NaCl	3.371(5)[b]	POT	p. w.
	1 M NaCl	3.37(3)[d]	POT	p. w.
	1 M NaCl	3.26(6)	POL	p. w.
	1 M NaCl	3.35(3)	POT	24
	1 M NaCl	3.24(3)	^{13}C NMR	25
	1 M $NaClO_4$	3.63(1)	POT	21
	1 M $NaClO_4$	3.48(18)	POT	22
	1 M $NaClO_4$	3.30(10)	POT	23
HGluc = γ-HGluc + H_2O	0.8 M NaCl	$\log K_{L,\gamma} = -0.68(2)$	NMR	p. w.
	not indicated	−0.59(6)	POL	19
	not indicated	−0.62	GC	29
HGluc = δ-HGluc + H_2O	$I \rightarrow 0$	$\log K_{L,\delta} = -0.95$	POL	20
	$I \rightarrow 0$	−0.81(9)	POT	21
	≈0.004 M[f]	−0.89	POL	28
	0.1 M $NaClO_4$	−0.91(6)	POT	21
	0.1 M $NaClO_4$	−0.54(12)[g]	^{13}C NMR	31
	0.5 M $NaClO_4$	−0.93(10)	POT	21
	0.8 M NaCl	−0.65(1)	^{13}C NMR	p. w.
	1 M $NaClO_4$	−1.15(6)	POT	21
	not indicated	−0.73(3)	POL	19
	not indicated	−0.67	GC	29
	not indicated	−0.66[g]	POL	30

[a] POT = potentiometry, POL = polarimetry, NMR = nuclear magnetic resonance spectroscopy, GC = gas chromatography using flame ionization detector.
[b] Protonation constant calculated by simultaneous curve fitting.
[c] Present work.
[d] Suggested protonation constant by individual curve fitting.
[e] Calculated from the rate coefficients determined at pH ≈ 2.
[f] Corresponds to pH = 2.36 reported by the authors; no additional background electrolyte was used.
[g] Data correspond to T = 20 [30] and 22 °C [31], respectively.

standard error (± 3 *SE*) as well as the literature data are presented in Table 1. For comparison, the same data for HGluc are also indicated.

By the rate law, one would expect higher reaction rate for lactonization with increasing aldonate concentration. To decide whether this reaction proceeded in the gulonate-containing systems or not, the curves were fitted individually as well. If the lactonization goes to completion, the apparent protonation constant ($K_{p,app}$) of the anion can be expressed as:

$$K_{p,app} = \frac{([HGul] + [\gamma\text{–}HGul] + [\delta\text{–}HGul])\cdot c}{[H^+][Gul^-]} = K_p(1 + K_{L,\gamma} + K_{L,\delta}) \quad (5)$$

It follows from eq. (5) that $K_{p,app} \geq K_p$ and only $K_{p,app}$ can be determined by potentiometry, since this method is not able to distinguish whether the pH change is the result of protonation or lactonization. The apparent constant would also be higher if the lactonization processes took place only partially (in this case K_L should to be replaced by the reaction quotient, Q_L). Each curve, however, yielded the same log $K_{p,app}$ values within 0.01 unit of uncertainty. Hence, K_p is independent from $[Gul^-]_{T,0}$ (*i.e.*, $K_{p,app} = K_p$), which means that our constants truly correspond to the protonation process.

The log K_p of Gul^- agrees fairly with the one obtained from NMR measurements at the same temperature and ionic strength [17], while the log K_p of $Gluc^-$ is in good agreement with literature data [22–25] obtained under the same experimental conditions, only the equilibrium constant given in Ref. [21] seems to be out to some extent.

Polarimetric measurements were also carried out with samples containing $[Gul^-]_T$ = 0.31 M; the corresponding optical rotation values are depicted in Fig. 2. Upon increasing $[HCl]_T$, the optical rotation of Gul^- markedly increases allowing the calculation of K_p. The observed rotation (α) depends on the concentrations of Gul^- and HGul by eq. (6):

$$\alpha = \alpha_{Gul^-}[Gul^-] + \alpha_{HGul}[HGul] \quad (6)$$

where $[Gul^-]$ and [HGul] are the corresponding actual concentrations (in mol·dm^{-3}) while α_{HGul} and α_{Gul-} are referred to as the products of the specific rotation and the path length (in °·dm^3·mol^{-1}).

Using the definition of K_p (eq. (1)) and the mass balance equation for Gul^-:

$$[Gul^-]_T = [Gul^-] + [HGul] \quad (7)$$

the rotation can be expressed as:

$$\alpha = [Gul^-]_T \frac{K_p 10^{-pH}\alpha_{HGul} + \alpha_{Gul^-}}{1 + K_p 10^{-pH}} \quad (8)$$

where $[Gul^-]_T$ is the total concentration of the anion. During calculations, the molar rotations of the two anions were held constant at the

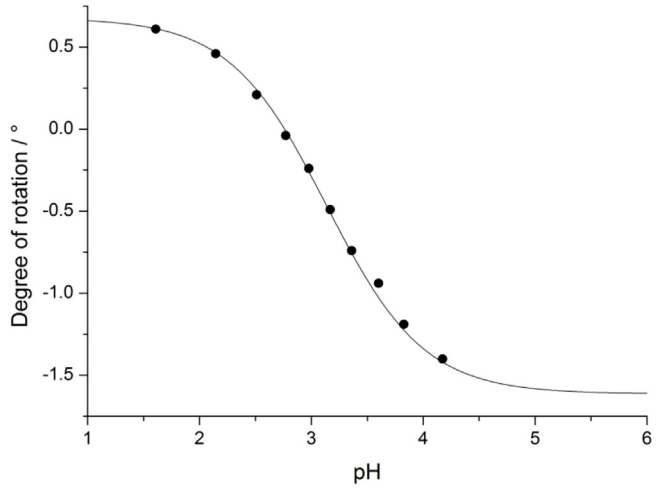

Fig. 2. Optical rotation of Na-L-gulonate (Gul^-) as a function of pH. Experimental conditions: T = (25 ± 2) °C, I = 1 M (NaCl); $[Gul^-]_T$ = 0.306 M and $[HCl]_T$ = 0.031–0.311 M. Symbols and solid lines represent the measured and calculated data, respectively.

value determined from calibrations. As a result, the *FP* was found to be 0.03°. The protonation constants obtained for the two aldonic acids are shown in Table 1.

Given that potentiometry is a more accurate method and is used more widely for the determination of protonation constants, the value of log K_p is suggested to be 3.33 for Gul^- as well as 3.37 for $Gluc^-$ (determined *via* titrations).

The main aim of these measurements was to determine the specific rotation of HGul. The calculated rotation was converted to traditional units yielding $[\alpha_{HGul}]$ = 5.7(8)°. For comparison, these measurements were undertaken for HGluc as well and $[\alpha_{HGluc}]$ was determined to be −5.7(8)°.

The specific rotation of HGluc at T = 20–25 °C was reported to be between −6.9° and 5.80° [19,20,28,30,45,46] pointing to the uncertainty of the experimental data. Nevertheless, −6.7° [45] and −6.9° [46] were determined from freshly prepared HGluc, which is close to our value and to those reported previously [19,30]. Based on this agreement, the specific rotation found for HGul seems to be reliable.

3.2. Lactonization equilibria for L-gulonic acid

Applying excess HCl, remarkable conversions of HGul and HGluc to their lactones were observed both in the ^{13}C and the ^{1}H NMR spectra. The latter spectra, however, were not well-resolved preventing their use from further quantitative analysis. The ^{13}C peaks of δ-HGul and γ-HGluc were assigned by comparing the spectra of the equilibrium solutions to those of γ-HGul and δ-HGluc (Figures S2 and S4) and to those of the anions [17,25]. A ^{13}C NMR spectrum for the equlibrium solution of HGul and its lactones is shown in Fig. 3.

It is clearly shown in Fig. 3 that γ-HGul is formed in the largest amount. The extreme stability of γ-HGul over δ-HGul reflects the qualitative rule that an *exo* double bond stabilizes the five-membered rings [27].

Although the actual structures of the acids and their lactones are rather different, the number of the O and H nuclei attached to the same carbon atom remains unaltered leading to similar relaxation times and hence, similar peak areas. Consequently, the sum of the integrals of C1–C6 may be applied for estimating the ratio of the actual concentrations within a certain spectrum. In addition to the well-separated peaks of the ^{13}C nuclei, (i) the position of the lactone signals is independent of the pH, (ii) the concentration of the free anion is negligible in excess of HCl, and (iii) this method does not require the exact value of pH (contrary to the determination of K_p). Moreover, this

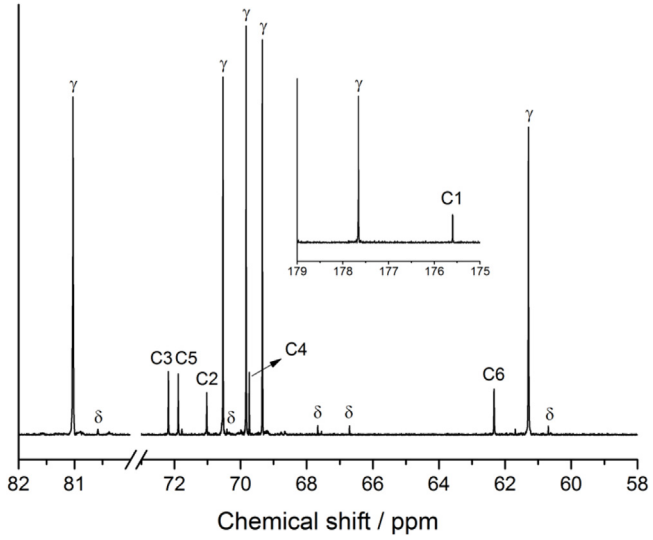

Fig. 3. ^{13}C NMR spectrum of L-gulonic acid (HGul) and its γ- and δ-lactones (γ-HGul and δ-HGul). Inset: region of carboxylate and carbonyl resonances. Experimental conditions: T = (25 ± 1) °C, I = 0.8 M (NaCl), 20% V/V D_2O; $[Gul^-]_T$ = 0.336 M, $[HCl]_T$ = 0.368 M. Peaks of HGul are labeled as C1–C6, while the γ and δ symbols correspond to the respective lactones.

method was successfully employed to determine the K_L of xylonic acid [47].

Based on these assumptions, $K_{L\gamma}$ and $K_{L,\delta}$ can be estimated by eqs. (9) and (10):

$$\frac{A_{\gamma-HGul}}{A_{HGul}} \approx \frac{[\gamma-HGul]}{[HGul]} = K_{L,\gamma} \quad (9)$$

$$\frac{A_{\delta-HGul}}{A_{HGul}} \approx \frac{[\delta-HGul]}{[HGul]} = K_{L,\delta} \quad (10)$$

where A is the respective total integrated area belonging to the acid or the lactones. It is seen in Fig. 3 that δ-HGul is formed to a very low extent. Thus, a reliable estimate for its concentration required long spectral acquisition (8192 scans), which was also applied for $Gluc^-$. The C1 (carbonyl) peak of δ-HGul did not appear even after such long accumulation time due to its long relaxation time.

The lactonization constants calculated this way are presented in Table 1, whilst the corresponding speciation diagrams are depicted in Fig. 4. The log $K_{L,\gamma}$ of HGul is in fair agreement with the one calculated

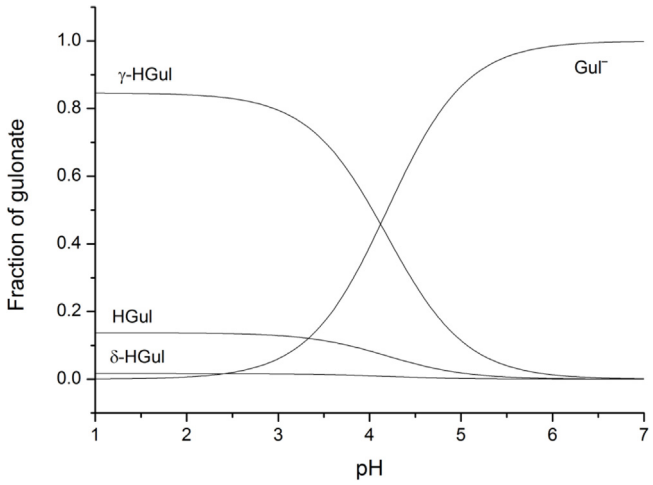

Fig. 4. Speciation diagram of the gulonate-related (Gul^-) species as a function of pH. The calculations correspond to T = 25 °C, I = 0.8–1.0 M and $[Gul^-]_T$ = 0.300 M.

from the rate coefficients given in Ref. [13]. On the other hand, the present value of log $K_{L,\delta}$ is lower by ≈ 0.4 units. Both literature data, however, are based on estimated initial reaction rates.

For the δ-lactonization constant of HGluc at $I > 0$, the deviation of our values from literature data is not higher than 0.2 logarithmic units (except for those given in Ref. [21]). Although our constants might be affected by the presence of 20% V/V D_2O, and the different ionic strength (0.8 M), the difference is lower than the scattering range of the reported values. Thus, the lactonization constants of HGluc given here are reliable at least at the semi-quantitative level which validates the constants of HGul, too.

The surprisingly high γ-lactonization constant of gulonic acid implies that this species should be considered when complexation equilibria between metal ions and Gul^- are studied in the acidic pH range. This is even more important when the metal ion is inert preventing the use fast potentiometric titrations.

3.3. Lactonization kinetics of L-gulonic acid

The applicability of ^{13}C NMR spectroscopy to characterize the lactonization reaction was utilized to find which lactone is formed faster. In a solution containing 0.34 M NaGul and 0.37 M HCl, the integrals of the different species were calculated, while the reaction was followed for a sufficiently long time (11 h). The six peaks of the acid and additional signals appeared 20 min after solution preparation. These peaks were at the same position as those of δ-HGul assigned during the equilibrium measurements (Fig. 3).

New signals appeared in the spectra after 27 min; these peaks were assigned as those of γ-HGul. The peak intensity of the six-membered lactone, however, increased faster compared to its isomer. Consequently, the formation of δ-HGul is faster in accordance with the observations of Levene and Simms [13].

Similarly to the lactonization constants, the reaction quotient at a given time (Q_L), can be estimated by the relative peak areas:

$$\frac{A_{\gamma-\mathrm{HGul},t}}{A_{\mathrm{HGul},t}} \approx \frac{[\gamma-\mathrm{HGul}]_t}{[\mathrm{HGul}]_t} = Q_{L,\gamma} \tag{11}$$

$$\frac{A_{\delta-\mathrm{HGul},t}}{A_{\mathrm{HGul},t}} \approx \frac{[\delta-\mathrm{HGul}]_t}{[\mathrm{HGul}]_t} = Q_{L,\delta} \tag{12}$$

These reaction quotients are plotted in Fig. 5. It is seen that after 4 h, $Q_{L,\delta}$ reaches a constant value (indicated with dashed line). This limiting value can be regarded as $K_{L,\delta}$ obtained from the ^{13}C spectra of the equilibrium solutions (Table 1). In conclusion, δ-HGul reaches the equilibrium rapidly, even when the reaction is far from the overall equilibrium state. Additionally, the assumption that δ-HGul would be an unstable intermediate can be ruled out.

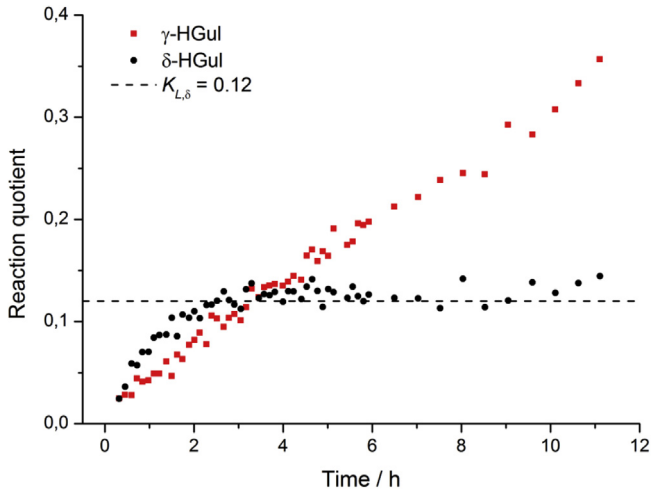

Fig. 5. Reaction quotients (Q_L) for L-gulonic acid (HGul) γ- and δ-lactones (γ-HGul and (δ-HGul) as a function of time. Experimental conditions: T = (25 ± 1) °C, I = 0.8 M (NaCl), 20% V/V D_2O; $[Gul^-]_T$ = 0.34 M, $[HCl]_T$ = 0.37 M. $Q_{\gamma,L}$ and $Q_{\gamma,L}$ were defined as the ratio of the total ^{13}C peak area of the lactone and that of HGul. The dashed line represents the δ-lactonization constant, $K_{L,\delta}$, determined from equilibrium measurements.

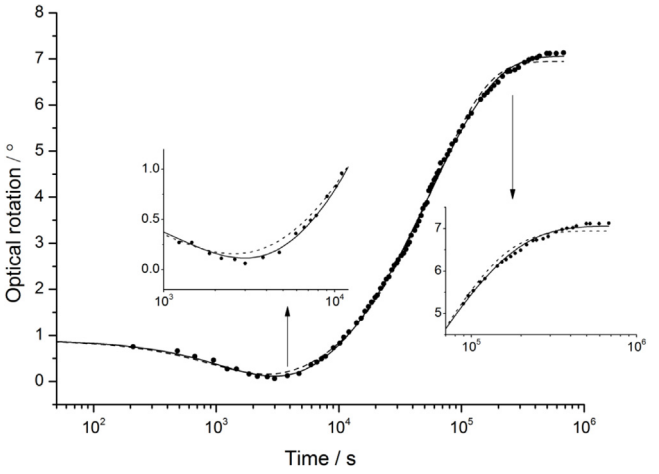

Fig. 6. Optical rotations for L-gulonic acid (HGul) as a function of time. Experimental conditions: T = (25 ± 2) °C, I = 1 M (NaCl); $[Gul^-]_T$ = 0.420 M, $[HCl]_T$ = 0.461 M. Full circles represent the measured points. Dashed line: calculated points by assuming reactions 13 and 17 (model 1); solid line: calculated points by assuming reactions 13, 17 and 21 (model 2). Insets: zoomed regions where model 1 shows the largest deviation from the experimental curve.

For HGluc, the calculation of rate constants was based on the observation that the equilibrium between HGluc and δ-HGluc is established long before the formation of γ-HGul [19]. Conversely, the present measurements (Fig. 5) provide spectroscopic evidence that this assumption is not applicable for the lactonization of HGul.

Despite the advantage of ^{13}C NMR in identifying the different lactones, accurate rate constants cannot be deduced due to the large uncertainty of the calculated integral ratios. Thus, polarimetry was chosen to study the kinetics of lactonization in detail, similarly to those published in the literature [13,14,19,20,28,30].

The optical rotation was recorded for the solution containing $[Gul^-]_T$ = 0.420 M and $[HCl]_T$ = 0.461 M; the measured points are plotted in Fig. 6. The initial rotation is positive according to the specific rotation of HGul. The measured values then decrease slightly due to the formation of the δ-lactone. During 10 days, the formation of γ-lactone exhibits a remarkable increase reaching a constant value of 7.1° implying the completion of the reaction. The direction of the overall change is the opposite but, expectedly, its shape is the same as that measured by Levene and Simms [13] for D-gulonic acid.

If the specific rotation of δ-HGul was positive, it would be even smaller than that of the acid, which is very unlikely for a cyclic compound. Hence, $[\alpha_{\delta\text{-HGul}}]$ is a large negative value, contrary to $[\alpha_{\gamma\text{-HGul}}]$. Since the nucleophilic OH group lies on the left (δ) or right (γ) side on the Fischer projection of HGul, δ-HGul is more levorotatory while γ-HGul is more dextrorotatory than the acid. Accordingly, L-gulonic acid obeys Hudson's lactone rule [15], hence, $[\alpha_{\delta\text{-HGul}}] < [\alpha_{\mathrm{HGul}}] < [\alpha_{\gamma\text{-HGul}}]$.

To model the experimental points, the acid-catalyzed lactonization and lactone hydrolysis for the two lactones were taken into account first.

$$\mathrm{HGul} + H_3O^+ = \delta-\mathrm{HGul} + H_3O^+ + H_2O \tag{13}$$

$$r_1 = k_1[H_3O^+][\mathrm{HGul}] \tag{14}$$

$$r_{-1} = k_{-1}[H_3O^+][\delta-\mathrm{HGul}] \tag{15}$$

Table 2
Reaction rate coefficients for the δ- and γ -lactonization (k_1, k_2, k_3) and the reverse hydrolysis reactions (k_{-1}, k_{-2}, k_{-3}) of L-gulonic acid (HGul) at T = (25 ± 2) °C and I = 1 M (NaCl). The coefficients were determined *via* polarimetry; ± 3 *SE* is given in parentheses.

Reaction[a]	Rate coefficient	Reference
HGul + H_3O^+ = δ-HGul + H_3O^+ + H_2O	$k_1 = 1.5(5)\cdot10^{-3}\,M^{-1}\,s^{-1}$	p. w.
	$k_{-1} = 1.3(3)\cdot10^{-2}\,M^{-1}\,s^{-1}$	p. w.
	$k_1^*[H_3O^+] = 3.1\cdot10^{-5}\,s^{-1}$[c]	13
	$k_{-1}^*[H_3O^+] = 9.6\cdot10^{-5}\,s^{-1}$[c]	13
HGul + H_3O^+ = γ-HGul + H_3O^+ + H_2O	$k_2 = 2.2(6)\cdot10^{-4}\,M^{-1}\,s^{-1}$	p. w.[b]
	$k_{-2} = 4.0(1.1)\cdot10^{-5}\,M^{-1}\,s^{-1}$	p. w.
	$k_2^*[H_3O^+] = 3.8\cdot10^{-6}\,s^{-1}$[c]	13
	$k_{-2}^*[H_3O^+] = 9.6\cdot10^{-7}\,s^{-1}$[c]	13
HGul + HGul = γ-HGul + HGul + H_2O	$k_3 = 2.5(8)\cdot10^{-5}\,M^{-1}\,s^{-1}$	p. w.
	$k_{-3} = 4.7(1.5)\cdot10^{-6}\,M^{-1}\,s^{-1}$	p. w.

[a] Excess H_3O^+ or HGul given in the reaction equation act as a catalyst.
[b] Present work.
[c] Data correspond to T = 25 °C. The pseudo-first order rate coefficients were determined at pH ≈ 2.

$$K_{L,\delta} = \frac{k_1}{k_{-1}} \tag{16}$$

$$\text{HGul} + H_3O^+ = \gamma\text{–HGul} + H_3O^+ + H_2O \tag{17}$$

$$r_2 = k_2[H_3O^+][\text{HGul}] \tag{18}$$

$$r_{-2} = k_{-2}[H_3O^+][\gamma\text{–HGul}] \tag{19}$$

$$K_{L,\gamma} = \frac{k_2}{k_{-2}} \tag{20}$$

H_3O^+ acts as a catalyst in these reactions (model 1) and its concentration was nearly constant (≈0.044 M, *i.e.*, $pH_c \approx 1.35$) throughout the reaction, because of employing excess HCl. During the fitting procedure, the protonation constant (from potentiometry) and the specific rotations (from polarimetry) were kept constant. Regarding the lactonization, the low conversion of δ-lactonization results in strong correlation between $K_{L,\delta}$ and the unknown [$\alpha_{\delta\text{-HGul}}$], thus, $K_{L,\delta}$ was fixed as well. On the other hand, $K_{L,\gamma}$ was treated as a parameter to be fitted.

The calculated rotations are depicted as dashed line in Fig. 6. The optical rotation could be appropriately fitted for the majority of the points, however, significant deviations remained around the minimum (≈3000 s) and from 10^5 s. By this model, the *FP* was found to be 0.09°.

Beside H_3O^+, HGul may also serve as a proton source. Hence, the decrease of its concentration would lead to the decrease of the reaction rate when significant amount of γ-HGul was formed at the expense of HGul. The model, therefore, was extended with the following reaction:

$$\text{HGul} + \text{HGul} = \gamma\text{–HGul} + \text{HGul} + H_2O \tag{21}$$

$$r_3 = k_3[\text{HGul}]^2 \tag{22}$$

$$r_{-3} = k_{-3}[\text{HGul}][\gamma\text{–HGul}] \tag{23}$$

$$K_{L,\gamma} = \frac{k_3}{k_{-3}} \tag{24}$$

The model augmented with eqs. (22)–(24) (model 2) provided satisfactory fitting for the whole duration of the reaction (solid line in Fig. 6) since the two ranges of systematic deviations disappeared. Accordingly, the *FP* decreased to 0.07°. The log $K_{L,\gamma}$ was found to be 0.73, 0.06 unit lower than the one obtained *via* ^{13}C NMR (log $K_{L,\gamma}$ = 0.79, Table 1). This difference is acceptable considering the different experimental methods and the overlapping uncertainty intervals of the two constants. Moreover, the specific rotation of δ-HGul was calculated to be −85(9)°.

The catalytic role of HGul is unusual, but can be explained by its high concentration (≈0.42 M). Similar concentration of HGul, 0.25 M, was applied by Levene and Simms [13], which is in the same order of magnitude; however, they analyzed only the initial reaction rates (preventing direct comparison of the rate coefficients). It is worth mentioning that the effect of self-catalysis can be observed near the minimum and at the end of the reaction. Hence, full curve fitting is necessary to pinpoint this process, as opposed to the method of initial rates.

Moreover, the self-catalysis for δ-HGul was taken into account, too, but the *FP* could not be lowered probably because of the low conversion of δ-lactonization. For HGluc, the direct interconversion of δ-HGul to γ-HGul (above pH ≈ 4) was proposed earlier [48–50]. It should be noted, however, that our ^1H and ^{13}C NMR spectra of the partially hydrolyzed δ-HGluc (in D_2O) showed the presence of HGluc but γ-HGluc was absent. Considering such reaction for the gulonate-containing system did not yield lower *FP*.

The rate coefficients obtained by assuming reactions (13), (17) and (21) are presented in Table 2. It is seen that both the rates of formation and hydrolysis are higher for δ-HGul. The ratio of k_1 and k_2 is 6.8, while the ratio of k_{-1} and k_{-2} is 325. Hence, the remarkable difference between $K_{L,\gamma}$ and $K_{L,\delta}$ does not arise from the rate of formation but from the rate of hydrolysis. Since the k_{-1}/k_{-2} parameter is closely related to the relative stability of the lactone rings, our finding supports quantitatively that an *exo* double bond stabilizes the γ-lactone ring [27].

Interestingly, the k_2 rate coefficient was found to be 8.6 times larger than k_3 inferring that the initial step of lactonization, which is the protonation of the carbonyl oxygen, proceeds much faster with H_3O^+ (H^+) than with HGul. In general acid catalysis the rate-determining step is the protonation of the COOH group. It follows from the mechanism that the stronger the acid the higher the reaction rate since H^+ can be released easier. HGul is weaker acid than H_3O^+, hence $k_3 < k_2$. (The same relation, $k_{-3} < k_{-2}$ holds for the reverse hydrolysis, too.) In conclusion, HGul is a general acid catalyst. Numerous general acid catalysts (*e.g.*, acetic acid, malonic acid) were studied for the hydrolysis of δ-HGluc in the work of Pocker and Green [20]. Our observations imply that if the aldonic acid is used in sufficiently high concentrations, it can catalyze its own lactonization and lactone hydrolysis processes.

3.4. The relative stability of L-gulonic acid γ- and δ-lactones

Previously, the equilibrium conformation of D-gulonic acid γ-lactone was studied by ^1H NMR spectroscopy [35]. Accordingly, γ-D-HGul favours the envelope conformation in equilibrium having the C2-OH group quasi-equatorially oriented. Additionally, the $HOCH_2$-CHOH side chain prefers the arrangement in which the interaction between the C5-OH and C3-OH groups is absent. Concerning the results of other aldopentono-lactones, the authors proposed that intramolecular hydrogen bonds play role in the stabilization of the preferred structures.

These observations are supported by the optimized structure of the enantiomer γ-L-HGul (Fig. 7). It is worth mentioning that the C=O

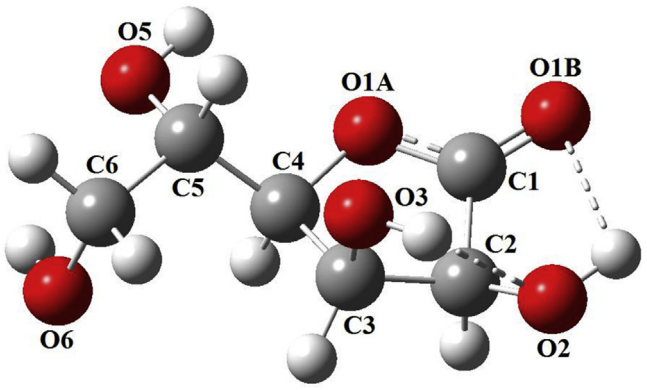

Fig. 7. Optimized structure of L-gulonic acid γ-lactone (γ-HGul). The calculations were performed at the M11/def2-TZVP level while solvent effects were taken into account by the CPCM model. The hydrogen bonds are visualized with dashed lines.

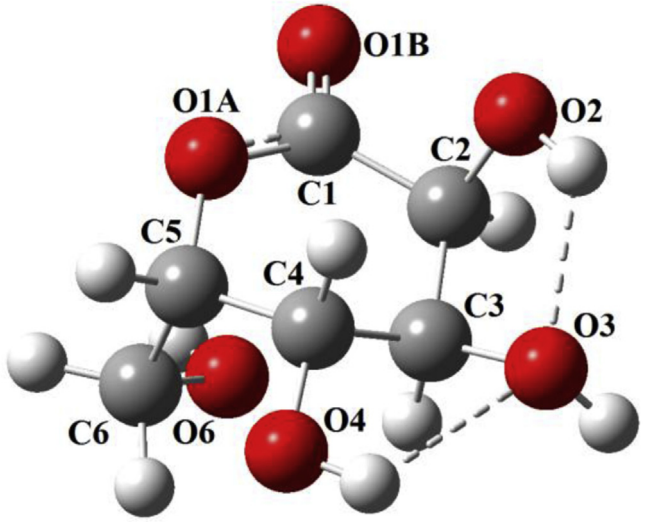

Fig. 8. Optimized structure of L-gulonic acid δ-lactone (δ-HGul). The calculations were performed at the M11/def2-TZVP level, while solvent effects were taken into account by the CPCM model. The hydrogen bonds are visualized by dashed lines.

double bond is partially delocalized through the O-C=O ester group. As a result, the C1-O1A bond length becomes shorter (1.333 Å) than the C4-O1A one (1.461 Å). The envelope conformation is slightly distorted as the dihedral angle for the C2-C1-O1A-C4 moiety is 5.81° instead of 0°.

Furthermore, two weak hydrogen bonds are formed between the O1B atom and the C2-OH group (2.373 Å) as well as between the C2-OH and C3-OH groups (2.178 Å).

Concerning δ-HGluc, the half-chair conformation was reported to prevail in aqueous medium [51], and it was deduced that the relative arrangement of C2-OH and C5-OH functions has a key role. That is, if these two groups are *trans*-disposed (*e.g.*, for δ-D-HGluc and δ-D-HGul), the half-chair, otherwise the boat conformation is favoured. This statement is confirmed by the present calculation for δ-L-HGul (Fig. 8), albeit its structure is somewhat distorted. That is, the dihedral angle for the C5-O1A-C2-C3 and the O1A-C1-C2-C3 moieties are −29.72° and 24.53° instead of 0°.

The lower thermodynamic stability of δ-HGul found by ^{13}C NMR spectroscopy was attested by these calculations, since δ-HGul has higher free energy by 26.1 kJ mol^{-1} at 298.15 K. (This is considerably higher than the energy of thermal motion, 2.5 kJ mol^{-1}) The delocalization of the π electrons again results that the C1-O1A bond length is shorter than the C4-O1A one (1.341 *vs.* 1.435 Å). The distances between C2-OH and C3-OH (2.043 Å) as well as between C4-OH and C3-OH (2.411 Å) indicate hydrogen bonding.

Conversely, there is no interaction between the C2-OH group and the carbonyl oxygen (O1B). This finding may in part explain the differences in the stability constants, but there can be other factors (*e. g.*, repulsion and overlapping orbitals), which affect the structure of the lactones. To disentangle all the interactions affecting the stability of these molecules would require more detailed computational analysis, which was beyond the scope of the present study.

4. Conclusions

Below pH = 5, the L-gulonate and the diastereomer D-gluconate undergo protonation as was attested by potentiometric and polarimetric measurements. The protonation constant was found to be very similar for the two anions as a token of structural similarity (*i.e.*, the number of OH groups). The basicity of the COO^- functional group, thus, is not significantly affected by the differences in the configuration of the OH groups. Moreover, this structural relationship yields the same absolute specific rotations for the acids formed upon protonation.

On the contrary, the relative arrangement of the individual OH groups along the carbon chain exhibits remarkable effect with regard to the stability of the five-membered (γ-HGul, γ-HGluc) and the six-membered lactones (δ-HGul, δ-HGluc) forming in acidic medium. The γ- and δ-lactonization constants of HGluc (log $K_{L,\gamma} = -0.68$, log $K_{L,\delta} = -0.65$) and the δ-lactonization constant of HGul (log $K_{L,\delta} = -0.91$) are small and similar in magnitude. Conversely, much higher stability was observed for γ-HGul having log $K_{L,\gamma}$ of 0.73–0.79.

Concerning the lactonization kinetics of gulonic acid, δ-HGul is formed faster, but the reverse hydrolysis of γ-HGul take place much slower, which appears to be the main reason of the higher stability of γ-HGul. The enhanced stability of the γ-lactone ring over its δ-isomer was confirmed by quantum chemical calculations, too.

Surprisingly, kinetic experiments show that beside H_3O^+, HGul can also act as a catalyst. In other words, HGul can catalyze its own γ-lactonization and the reverse hydrolysis if applied in sufficiently high concentrations. In this respect, gulonic acid probably behaves as a general acid catalyst similarly to other weak acids, which were studied previously concerning the lactonization processes of gluconic acid. The existence of this self-catalysis implies that such reactions may take place for other aldonic acids as well.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carres.2018.07.006.

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